

Labeled in Matthew 2004 64/019
PCT

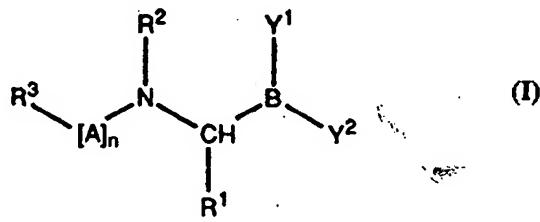
WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5: A61K 37/02	A1	(11) International Publication Number: WO 94/25049 (43) International Publication Date: 10 November 1994 (10.11.94)
(21) International Application Number: PCT/US94/04058		(81) Designated States: AU, CA, CZ, FI, HU, JP, KR, NO, NZ, PL, SK, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).
(22) International Filing Date: 21 April 1994 (21.04.94)		
(30) Priority Data: 08/052,835 27 April 1993 (27.04.93) US 08/204,055 2 March 1994 (02.03.94) US		Published With international search report.
(71) Applicant: THE DU PONT MERCK PHARMACEUTICAL COMPANY [US/US]; 1007 Market Street, Wilmington, DE 19898 (US).		
(72) Inventors: FEVIG, John, Matthew; 987 Church Road, Lincoln University, PA 19352-9349 (US). KETTNER, Charles, Adrian; 2411 Chatham Drive, Wilmington, DE 19803-2709 (US). LEE, Sheng-Lian, O.; 3304 North Rockfield Drive, Wilmington, DE 19810-3221 (US). CARINI, David, John; 1921 Julian Road, Wilmington, DE 19803-3107 (US).		
(74) Agents: REINERT, Norbert, F. et al.; The Du Pont Merck Pharmaceutical Company, Legal/Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US).		

(54) Title: AMIDINO AND GUANIDINO SUBSTITUTED BORONIC ACID INHIBITORS OF TRYPSIN-LIKE ENZYMES



(57) Abstract

This invention relates to Novel α -aminoboronic acid and corresponding peptide analogs of formula (I) are disclosed.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

Title

Amidino and Guanidino Substituted Boronic Acid
Inhibitors of Trypsin-Like Enzymes

5

Cross Reference to Related Applications

This application is a continuation-in-part of
Application Serial Number 08/052,835, filed April 27,
1993.

10

Field of the Invention

The present invention relates generally to α -
aminoboronic acids and corresponding peptide analogs in
which the alpha substituent is either an aromatic
guanidino, isothiouronium, amidino group, halogen, cyano
15 group or an aliphatic amidino, isothiouronium, or
formamidino group.

Background of the Invention

Simple boronic acids are inhibitors of serine
20 proteases. For example, Koehler et al. *Biochemistry* 10: 2477 (1971) reports that 2-phenylethane boronic acid
inhibits chymotrypsin at millimolar levels. The
synthesis of boronic acid analogs of N-acyl- α -amino
acids has yielded more effective inhibitors. Ac-
25 boroPhe-OH, R-1-acetamido-2-phenylethane boronic acid,
inhibits chymotrypsin with a K_i of 4 μ M Matteson et al.
J. Am. Chem. Soc. 103: 5241 (1981). More recently,
Shenvi, US 4,537,773 (1985) disclosed that boronic acid
30 analogs of α -amino acids, containing a free amino group,
were effective inhibitors of aminopeptidases. Shenvi,
US 4,499,082 (1985) discloses that peptides containing
an α -aminoboronic acid with a neutral side chain were
more effective inhibitors of serine proteases exceeding
inhibitors disclosed earlier by as much as 3 orders of
35 magnitude in potency. The chemistry of α -aminoboronic
acids was further expanded to the synthesis of peptide

analogs containing boronic acid with positive charged sidechains, boroLysine, boroArginine, boroOrnithine, and isothiouronium analogs (EPA 0 293 881, 12/7/88). This series of compounds have provided highly effective

5 inhibitors of thrombin and other trypsin-like enzymes.

The boroArginine analogs specifically designed as

thrombin inhibitors are highly effective in the

inhibition of blood coagulation both *in vitro* and *in vivo*. In the present invention, this group of compounds

10 is extended to aliphatic amidino and formamidino, to aromatic amidino and guanidino, and to cyano and halogen substituted aromatic boronic acid analogs.

It should be noted that additional boronic acids have been disclosed. Metternich (EP 0471651) have

15 described peptides containing boroArginine and boroLysine which contain at least one unnatural amino acid residue. Elgendi et al. *Tetrahedron Lett.*, 33, 4209-4212 (1992) have described peptides containing α -aminoboronic acids with aliphatic neutral sidechains

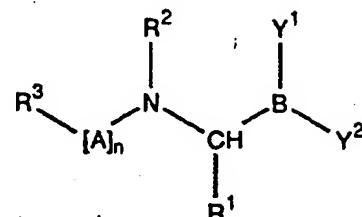
20 which are thrombin inhibitors. Kakkar in (WO 92/07869) has claimed peptide thrombin inhibitors of the general structure, $X-Aa_1-Aa_2-NH-CH(Y)-Z$ where Aa_1 and Aa_2 are unnatural amino acid residues. Z is $-CN$, $-COR$, $-B(R^2)(R^3)$, $-P(O)(R)(R)$, and Y is $-[CH_2]_n-Q$ or $-CH_2-Ar-Q$

25 where $Q = H$, amino, amidino, imidazole, guanidino or isothioureido and $n=1-5$ and where R_2 and R_3 are the same or different and are selected from the group consisting of OH , OR^6 , and NR^6R^7 , or R^2 and R^3 taken together represent the residue of a diol. This specialized group

30 of compounds where Z is $-B(R^2)(R^3)$ fall within the scope of our present application. It should be noted that this is a narrow subset of Kakkar et al. However, rather specialized chemical transformations are required to prepare these compounds and Kakkar et al. does not make an enabling disclosure.

Summary of the Invention

A compound of formula (I)



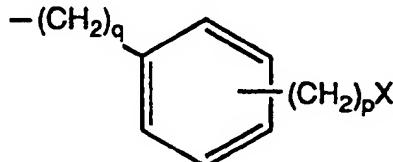
I

5 wherein

R1 is

a) C1-C12-alkyl substituted with -CN, -C(NH)NHR⁶,
 -NHC(NH)H, -NHC(NH)NHR⁶, -SC(NH)NHR⁶, -NHC(NH)NHOH,
 -NHC(NH)NHCN, -NHC(NH)NHCOR⁶, or

10 b)



X is

a) halogen (F, Cl, Br, I)
 b) -CN,
 15 c) -NO₂,
 d) -CF₃,
 e) -NH₂
 f) -NHC(NH)H,
 g) -NHC(NH)NHOH,
 20 h) -NHC(NH)NHCN,
 i) -NHC(NH)NHR⁶,
 j) -NHC(NH)NHCOR⁶,
 k) -C(NH)NHR⁶,
 l) -C(NH)NHCOR⁶,
 25 m) -C(O)NHR²,
 n) -CO₂R²,
 o) -OR², or
 p) -OCF₃

q) $-\text{SC}(\text{NH})\text{NHR}^6$;

R^2 is

a) H,

b) C1-C4-alkyl,

5 c) aryl, wherein aryl is phenyl or napthyl
optionally substituted with one or two substituents
selected from the group consisting of halo (F, Cl,
Br, I), C1-C4-alkyl, C1-C4-alkoxy, $-\text{NO}_2$, $-\text{CF}_3$,
 $-\text{S}(\text{O})_r\text{C1-C4-alkyl}$, $-\text{OH}$, $-\text{NH}_2$, $-\text{NH}(\text{C1-C4-alkyl})$,
10 $-\text{N}(\text{C1-C4-alkyl})_2$, $-\text{CO}_2\text{R}^4$, or

d) $-\text{C1-C4-alkylaryl}$, where aryl is defined above;

R^3 is H, alkyl, aryl, alkylaryl, or an NH_2 -blocking
group comprised of 1-20 carbon atoms;

R^4 and R^5 are independently

15 a) H,

b) C1-C4-alkyl, or

c) $-\text{CH}_2\text{-aryl}$, where aryl is defined above;

R^6 is

a) H,

20 b) C1-C4-alkyl,

c) aryl, wherein aryl is phenyl or napthyl
optionally substituted with one or two substituents
selected from the group consisting of halo (F, Cl,
Br, I), C1-C4-alkyl, C1-C7-alkoxy, $-\text{NO}_2$, $-\text{CF}_3$,

25 $-\text{S}(\text{O})_r\text{C1-C4-alkyl}$, $-\text{OH}$, $-\text{NH}_2$, $-\text{NH}(\text{C1-C4-alkyl})$,
 $-\text{N}(\text{C1-C4-alkyl})_2$, $-\text{CO}_2\text{R}^4$, or

d) $-\text{C1-C4-alkylaryl}$, where aryl is defined above;

A is an amino acid residue or a peptide comprised of 2-
20 amino acid residues;

30 y^1 and y^2 are

a) $-\text{OH}$,

b) $-\text{F}$,

c) C1-C8-alkoxy, or

when taken together y^1 and y^2 form a

d) cyclic boron ester where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-3 heteroatoms which can be N, S, or O,

n is 0 or 1;

5 p is 0 to 3;

q is 0 to 4;

r is 0 to 2;

and pharmaceutically acceptable salts thereof, with the proviso that when R¹ is aliphatic, an R⁶ substituent on

10 -NHC(NH)NHR⁶ cannot be H.

Preferred are those compounds of formula (I) where Y¹ and Y² are

a) -OH,

when taken together Y¹ and Y² form a

15 b) cyclic boron pinacol ester, or

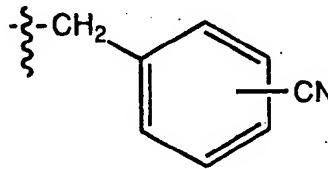
c) cyclic boron pinanediol ester;

R¹ is

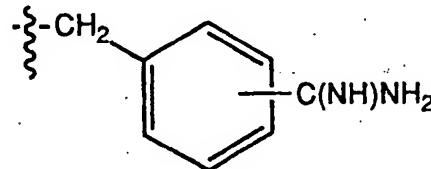
a) -(CH₂)₃NHC(NH)H,

b) -(CH₂)₄C(NH)NH₂,

20 c)

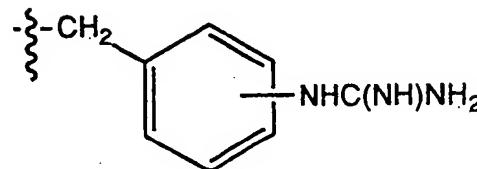


d)



, or

e)



R^2 is H;
 A is Pro or (D)Phe-Pro;
 R^3 is
 a) H,
 5 b) Boc,
 c) Z, or
 d) Ac, or
 e) hydrocinnamoyl
 f) C1-C10 alkyl sulfonyl
 10 g) C1-C15 alkylaryl sulfonyl

Illustrative of the preferred compounds of this invention are the following:

15 • Ac-(D)Phe-Pro-NH-CH[(CH₂)₄CN]BO₂-C₁₀H₁₆
 • Ac-(D)Phe-Pro-NHCH[(CH₂)₄C(NH)NH₂]BO₂-C₁₀H₁₆
 • Ac-(D)Phe-Pro-NHCH[(CH₂)₃-NHC(NH)H]B(OH)₂
 • Boc-(D)Phe-Pro-NHCH[(CH₂)₃-NHC(NH)H]B(OH)₂.
 • Ac-(D)Phe-Pro-boroPhe[m-C(NH)NH₂]-C₁₀H₁₆
 20 20 • Ac-(D)Phe-Pro-boroPhe(m-CH₂NH₂)-C₁₀H₁₆
 • Ac-(D)Phe-Pro-boroPhe(m-Br)-C₁₀H₁₆
 • Ac-(D)Phe-Pro-boroArg(CN)-C₁₀H₁₆
 • Ac-(D)Phe-Pro-boroPhe(p-CN)-C₁₀H₁₆
 • Boc-(D)Phe-Pro-boroPhe-(m-CN)-C₁₀H₁₆
 25 25 • N,N-(CH₃)₂-(D)Phe-Pro-boroPhe-(m-CN)-OH•HCl (ISOMER I)
 • Ac-(D)Phe-Pro-boroPhe-(m-CN)-OH•HCl
 • Ms-(D)Phe-Pro-boroPhe-(m-CN)-OH•HCl
 • Boc-(D)Thiazolylalanine-Pro-boroPhe-(m-CN)-C₁₀H₁₆
 30 30 • Boc-(D)3-Pyridylalanine-Pro-boroPhe-(m-CN)-C₁₀H₁₆
 • Ms-(D)3-Pyridylalanine-Pro-boroPhe-(m-CN)-C₁₀H₁₆
 • Boc-(D)2-Pyridylalanine-Pro-boroPhe-(m-CN)-C₁₀H₁₆
 • Boc-(D)2-Thienylalanine-Pro-boroPhe-(m-CN)-C₁₀H₁₆
 • Ms-(D)2-Thienylalanine-Pro-boroPhe-(m-CN)-C₁₀H₁₆
 35 35 • Boc-(D)Phe-Aze-boroPhe-(m-CN)-C₁₀H₁₆
 • Hydrocinnamoyl-Pro-boroIrg(CH₃)-OH•HBr

- Ac-(D)Phe-Pro-boroArg(CH₃)-OH•HCl
- PhCH₂SO₂-(D)Phe-Pro-boroOrn(CH=NH)-OH•HCl
- CH₃CH₂CH₂SO₂-(D)Phe-Pro-boroOrn(CH=NH)-OH•HCl
- CH₃CH₂CH₂SO₂-(D)Phe-Pro-boroArg(CH₃)-OH•HCl
- 5 • Ac-(D)Phe-Sar-boroOrn(CH=NH)-OH•HCl
- Boc-(D)Phe-Sar-boroPhe(mCN)-C₁₀H₁₆
- Boc-(D)Phe-Aze-boroOrn(CH=NH)-OH•HCl
- 4-(Phenyl)benzoyl-boroOrn(CH=NH)-C₁₀H₁₆•HCl

10 This invention also provides compositions comprising one or more of the foregoing compounds and methods of using such compositions in the treatment of aberrant proteolysis such as thrombosis in mammals or as reagents used as anticoagulants in the processing of
15 blood to plasma for diagnostic and other commercial purposes.

Detail Description of the Invention

As used throughout the specifications, the
20 following abbreviations for amino acid residues or amino acids apply:

Ala	=	L-alanine
Arg	=	L-arginine
Asn	=	L-asparagine
25	Asp	= L-aspartic acid
	Aze	= azedine-2-carboxlic acid
	Cys	= L-cysteine
	Gln	= L-glutamine
	Glu	= L-glutamic acid
30	Gly	= glycine
	His	= L-histidine
	Homolys	= L-homolysine
	Ile	= L-isoleucine
	Irg	= isothiouronium analog of L-Arg
35	Leu	= L-leucine
	Lys	= L-lysine

Met	=	L-methionine
Orn	=	L-ornithine
Phe	=	L-phenylalanine
Pro	=	L-proline
5	Ser	= L-serine
	Thr	= L-threonine
	Trp	= L-tryptophan
	Tyr	= L-tyrosine
	Val	= L-valine
10	Sar	= L-sarcosine
	Phe(4-fluoro)=	para-fluorophenylalanine

The "D" prefix for the foregoing abbreviations indicates the amino acid is in the D-configuration.

15 "D,L" indicates the amino is present in mixture of the D- and the L-configuration. The prefix "boro" indicates amino acid residues where the carboxyl is replaced by a boronic acid or a boronic acid ester. For example, if R¹ is isopropyl and Y¹ and Y² are OH, the C-terminal residue is abbreviated "boroVal-OH" where "-OH" indicates the boronic acid is in the form of the free acid. The pinanediol boronic acid ester and the pinacol boronic acid ester are abbreviated "-C₁₀H₁₆" and "-C₆H₁₂", respectively. Examples of other useful diols 20 for esterification with the boronic acids are 1,2-ethanediol, 1,3-propanediol, 1,2-propanediol, 2,3-butanediol, 1,2-diisopropylethanediol, 5,6-decanediol, and 1,2-dicyclohexylethanediol. The formamidino modified amino group is abbreviated (CH=NH). 25 For example, the formamidino analog of -boroOrn-OH {-NH-CH[(CH₂)₃-NH-CH(NH)H]B(OH)₂} is -boroOrn(CH=NH)-OH. Analogs containing sidechain substituents are described by indicating the substituent in parenthesis following the name of the parent residue. For example the analog 30 of boroPhenylalanine containing a meta cyano group is -boroPhe(mCN)-. N-alkyl substituents on the guanidino 35

group of boroArg- or on the isothiouronium analogs (boroIrg) are also put in parenthesis in a similar manner. Other abbreviations are: Z, benzyloxycarbonyl; BSA, benzene sulfonic acid; THF, tetrahydrofuran; Boc-,

5 t-butoxycarbonyl-; Ac-, acetyl; pNA, p-nitro-aniline; DMAP, 4-N,N-dimethylaminopyridine; Tris, Tris(hydroxymethyl)aminomethane; MS, mass spectrometry; FAB/MS, fast atom bombardment mass spectrometry; LRMS(NH₃-CI) and HRMS(NH₃-CI) are low and high 10 resolution mass spectrometry, respectively, using NH₃ as an ion source.

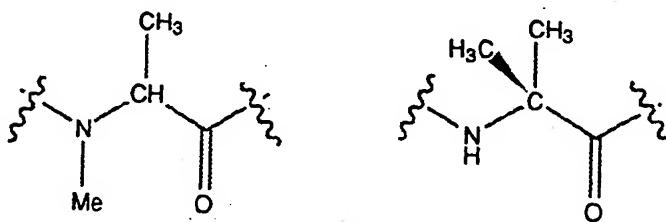
It is understood that many of the compounds of the present invention contain one or more chiral centers and that these stereoisomers may possess distinct physical

15 and biological properties. The present invention comprises all of the stereoisomers or mixtures thereof. If the pure enantiomers or diasteromers are desired, they may be prepared using starting materials with the appropriate stereochemistry, or may be separated from 20 mixtures of undesired stereoisomers by standard techniques, including chiral chromatography and recrystallization of diastereomeric salts.

"NH₂-blocking group" as used herein, refers to various acyl, thioacyl, alkyl, sulfonyl, phosphoryl, and 25 phosphinyl groups comprised of 1 to 20 carbon atoms. Substitutes on these groups maybe either alkyl, aryl, alkylaryl which may contain the heteroatoms, O, S, and N as a substituent or as inchain component. A number of NH₂-blocking groups are recognized by those skilled in 30 the art of organic synthesis. By definition, an NH₂-blocking group may be removable or may remain permanently bound to the NH₂. Examples of suitable groups include formyl, acetyl, benzoyl, trifluoroacetyl, and methoxysuccinyl; alkyl and alkylaryl sulfonyl, 35 groups, such as n-propylsulfonyl, phenylmethyl and benzylsulfonyl; aromatic urethane protecting groups,

such as, benzyloxycarbonyl; and aliphatic urethane protecting groups, such as t-butoxycarbonyl or adamantlyloxycarbonyl. Gross and Meinhoffer, eds., *The Peptides*, Vol 3; 3-88 (1981), Academic Press, New York, 5 and Greene and Wuts *Protective Groups in Organic Synthesis*, 315-405 (1991), J. Wiley and Sons, Inc., New York disclose numerous suitable amine protecting groups and they are incorporated herein by reference for that purpose.

10 "Amino acid residues" as used herein, refers to natural or unnatural amino acids of either D- or L- configuration. Natural amino acids residues are Ala, Arg, Asn, Asp, Aze, Cys, Gln, Glu, Gly, His, Ile, Irg Leu, Lys, Met, Orn, Phe, Phe(4-fluoro), Pro, Sar, Ser, 15 Thr, Trp, Tyr, and Val. Roberts and Vellaccio, *The Peptides*, Vol 5; 341-449 (1983), Academic Press, New York, discloses numerous suitable unnatural amino acids and is incorporated herein by reference for that purpose. Additionally, said reference describes, but 20 does not extensively list, acyclic N-alkyl and acyclic α,α -disubstituted amino acids. Included in the scope of the present invention are N-alkyl, aryl, and alkylaryl analogs of both in chain and N-terminal amino acid residues. Similarly, alkyl, aryl, and alkylaryl maybe 25 substituted for the alpha hydrogen. Illustrated below are examples of N-alkyl and alpha alkyl amino acid residues, respectively.



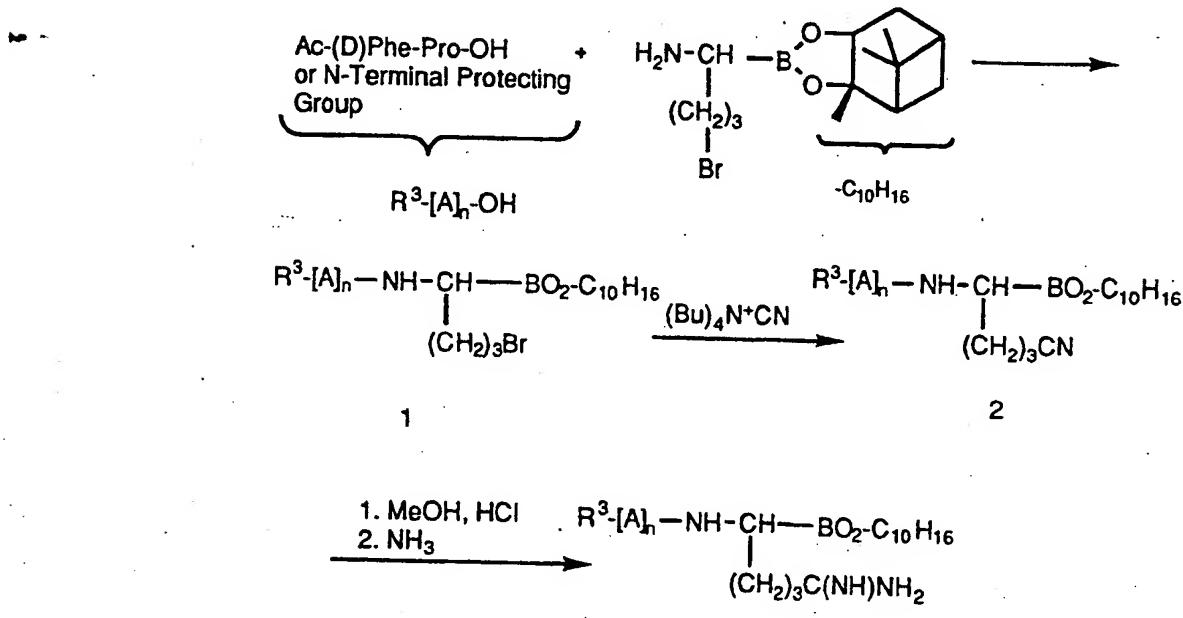
"Amino acids residues" also refers to various amino acids where sidechain functional groups are coupled with appropriate protecting groups known to those skilled in the art. "The Peptides", Vol 3, 3-88 (1981) discloses 5 numerous suitable protecting groups and is incorporated herein by reference for that purpose.

Synthesis

Novel peptide boronic acids containing aliphatic 10 sidechains were prepared by the series of reactions outlined in Scheme I. First, the precursor, $\text{NH}_2\text{-CH}[(\text{CH}_2)_n\text{Br}]\text{BO}_2\text{-C}_{10}\text{H}_{16}$, $n = 3$ or 4 , was prepared and coupled with an N-terminal protecting group or with an N-terminal and sidechain protected peptide by the 15 procedure we have described previously [Kettner et al. *J. Biol. Chem.* **265** 18289-18297 (1990)]. An example of this product is **1** where the above intermediate is coupled to Ac-(D)Phe-Pro-OH . **1** was converted to the corresponding alkyl cyanide **2** by treatment with 20 tetrabutyl ammonium cyanide in THF at 55°C for 2 hours. This appears to be a general method for introducing the cyano group. In contrast, other common methods of introducing this group can be applied only with limited success. For example, the reaction of $\text{Ac-(D)Phe-Pro-NH-CH}[(\text{CH}_2)_4\text{-Br}]\text{BO}_2\text{-C}_{10}\text{H}_{16}$ with KCN in $\text{N,N-dimethylformamide}$ failed to yield a detectable product. Our data are consistent with the formation of a cyclic 25 product arising from the nucleophilic displacement of the sidechain bromide by the adjacent amide NH . Treatment of $\text{Z-NH-CH}[(\text{CH}_2)_4\text{-Br}]\text{BO}_2\text{-C}_{10}\text{H}_{16}$ with NaCN in $\text{N,N-dimethylformamide}$ gave the cyano compound, but only 30 in low yield, indicating that cyclization does not occur quite so readily when the urethane protecting group (Z) is present. Typically, **2** was purified by standard 35 techniques such as silica gel chromatography. The corresponding amidine, **3**, was prepared by treating the

nitrile with a saturated solution of a mineral acid such as HCl in methanol. Excess solvent and acid were removed by evaporation and the residue was allowed to react with anhydrous ammonia to yield the desired 5 product.

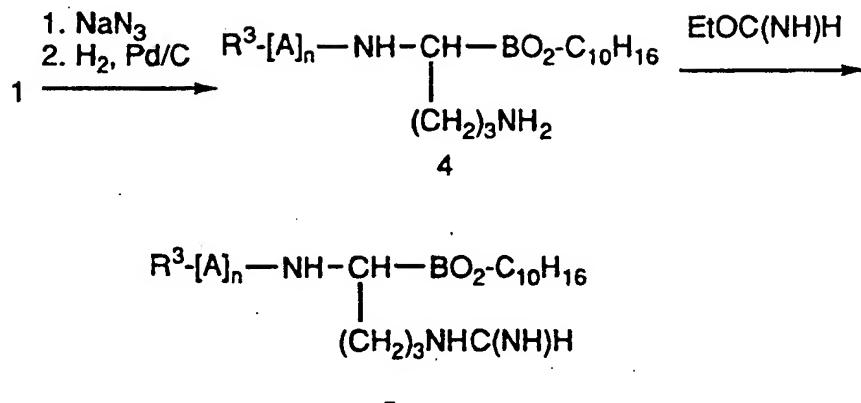
Scheme 1



The formamidino substituted boronic acid, 5, was prepared by the synthesis of the corresponding alkyl amine such as Ac-(D)Phe-Pro-boroOrn-C₁₀H₁₆ 4, Scheme 2.

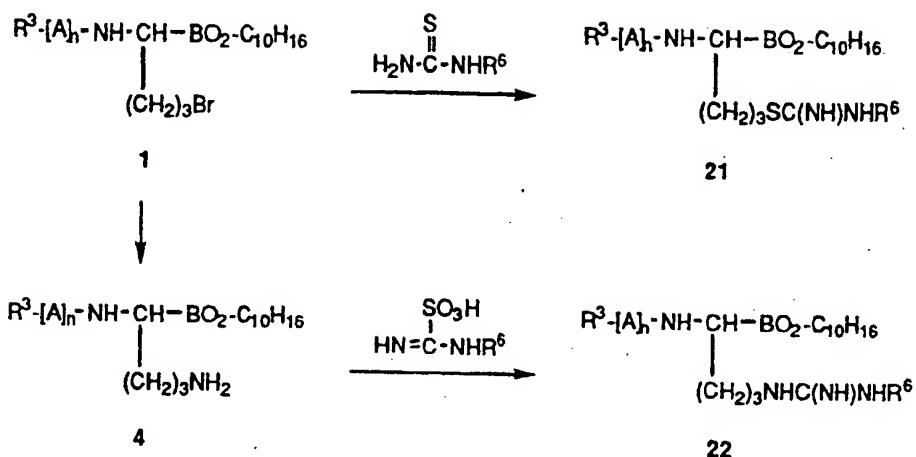
10 This in turn was prepared by treating 1 with sodium azide followed by hydrogenation (Kettner et al., 1990). The amine, 4, was treated with ethyl formimidate to yield the formamidino compound, 5.

Scheme 2



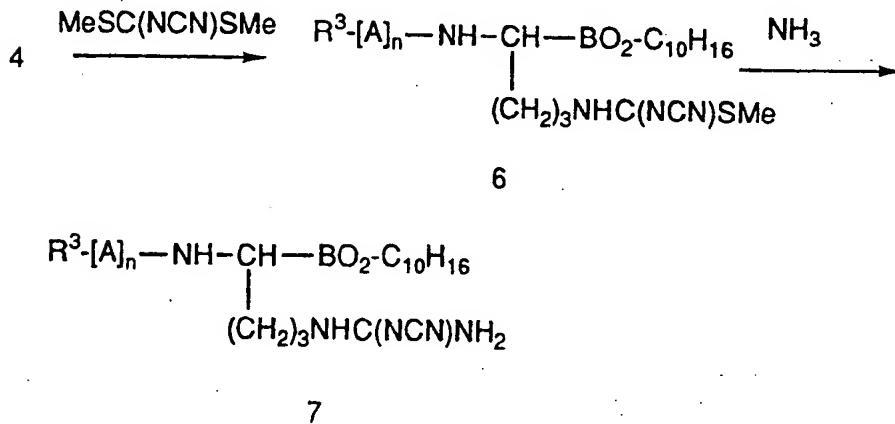
N-substituted isothiouronium derivatives and N-substituted guanidines are readily prepared as shown in Scheme 2a. Treatment of bromide 1 with a thiourea 5 produces directly the isothiouronium 21. Alternatively 1 can be converted to the amine 4 as shown in Scheme 2. Employing a method originally described by Kim et al., *Tetrahedron Lett.* 29, 3183 (1988), the amine 4 then is heated with a formamidinesulfonic acid in the presence of 4-DMAP to afford the guanidine 22. The required formamidinesulfonic acids can be prepared by oxidation of the corresponding thioureas, see: Walter and Randau, *Liebigs Ann. Chem.* 722, 98 (1969).

Scheme 2a



The substituted boronic acid, 1, is prepared by treating 4 with dimethyl cyanodithioiminocarbonate or 5. diphenyl cyanodicarbonimide to yield the S-methyl isourea (6) or O-phenyl isourea, respectively, using a procedure similar to that reported by Barpill et al. *J. Heterocyclic Chem.* 25, 1698 (1988), Scheme 3. This intermediate is treated with ammonia in either THF or 10 alcohol to yield the desired product.

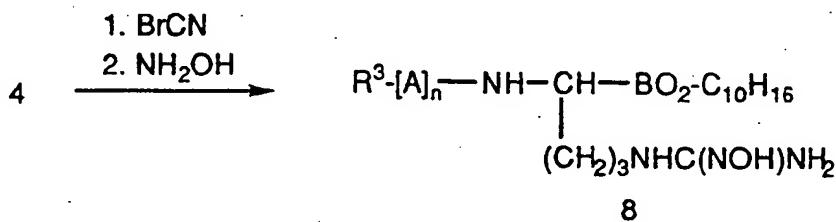
Scheme 3



Hydroxyguanidino inhibitors are prepared by treating **4** with cyanogen bromide or cyanogen chloride followed by hydroxylamine to yield **8**, Scheme 4. These are known chemical transformations, Nakahara et. al.

5 *Tetrahedron*, **33**, 1591 (1977) and Belzecki et al. *J. Chem. Soc. Chem. Commun.*, 806 (1970).

Scheme 4.

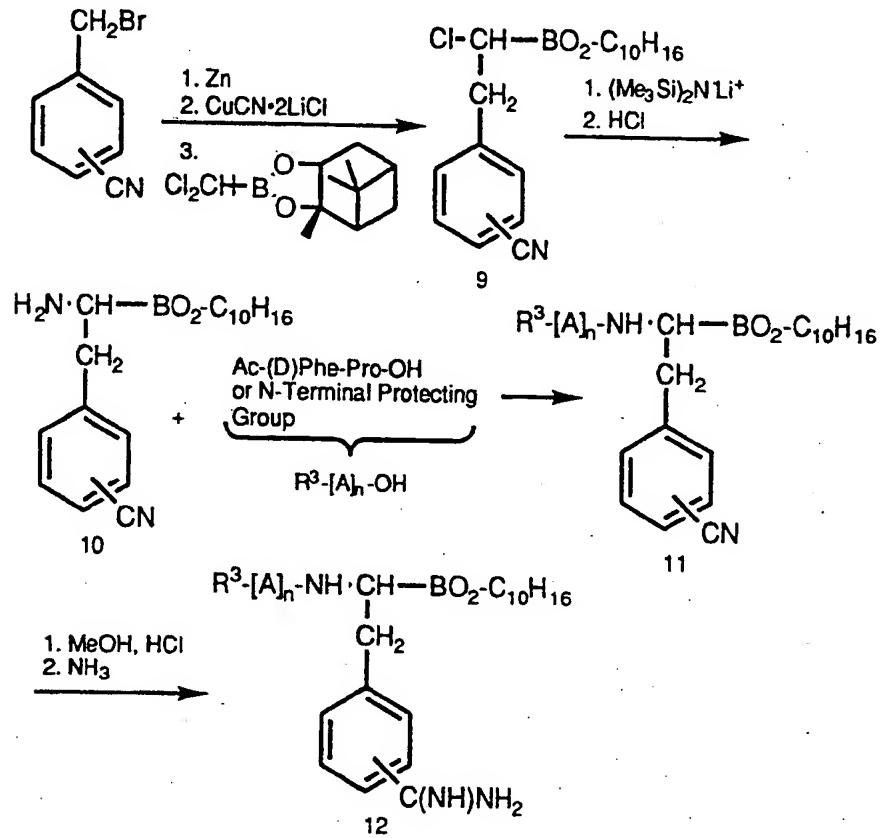


10

The preparation of new aromatic boronic acids are shown in Scheme 5. Functionalized benzylic anions containing either a halogen or cyano substituent (the cyano group is shown for illustration) are obtained by 15 treatment with activated Zn metal in THF or other inert solvent and then with CuCN·2LiCl [Berk et al. *Organometallics* **9**, 3053-3064 (1990)]. Dichloromethyl boronic acid pinanediol was prepared by the method described by Tsai et al. *Organometallics* **2**, 1543-1545 20 (1983). It was allowed to react with the transmetalated anion to yield **9**. This was the only acceptable method of preparing these functionalized benzylic anions. For example, treatment of *p*-nitobenzyl chloride with lithium metal using the method of Michel et al. *J. Organometallic Chem.* **204**, 1-12 (1981) failed to yield an identifiable product. Similarly, treatment of *p*-cyanobenzyl chloride with lithium naphthalenide in the presence of ZnCl₂ using the conditions of Zhu et al. *J. Org. Chem.* **56**, 1445-1453 (1991) did not give the desired 30 product.

The α -aminoboronic acid, 10, was obtained by treating 9 with the lithium salt of hexamethyldisilazane and removing the trimethylsilyl groups by treatment with anhydrous HCl. 10 was coupled to either an N-terminal protecting group or to a peptide using known techniques.

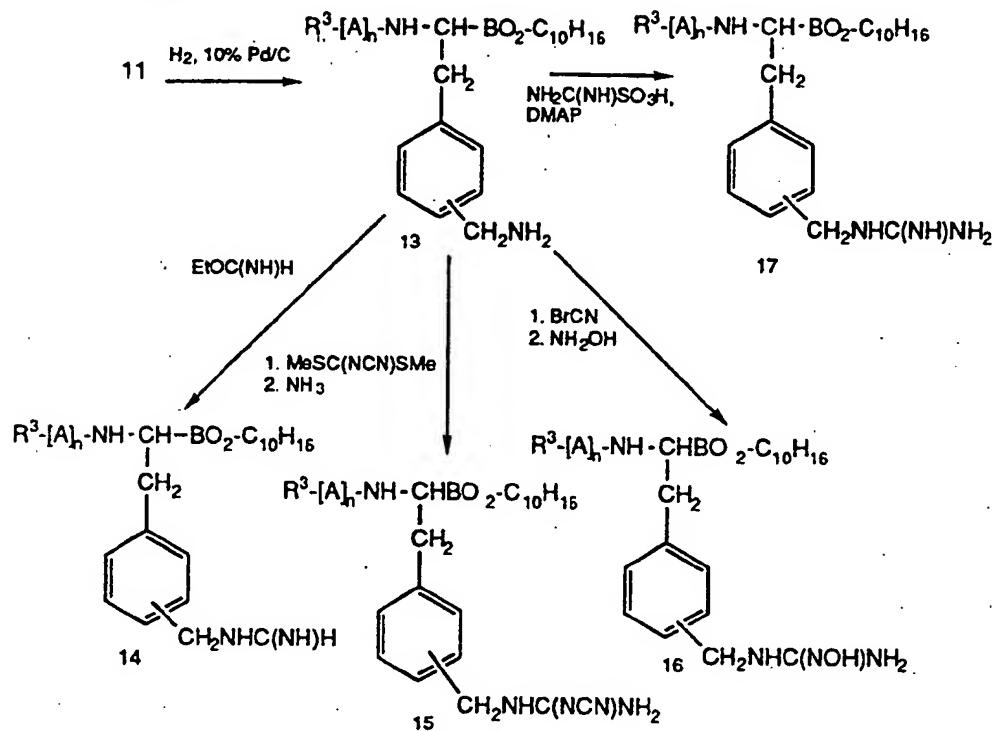
The aromatic substituted cyanides, 11, were converted to the corresponding amidino compound, 12, using the same sequence of reactions described for preparation of the aliphatic amidino compound, 3. Scheme 5



11 can be hydrogenated to yield the corresponding aminomethyl group as an aromatic substituent 13, Scheme 6. The corresponding formamidino, cyanoguanidino, 15 hydroxyguanidino and guanidino compounds, 14, 15, 16,

and 17, respectively, are prepared by the procedures described for the aliphatic series, Scheme 1.

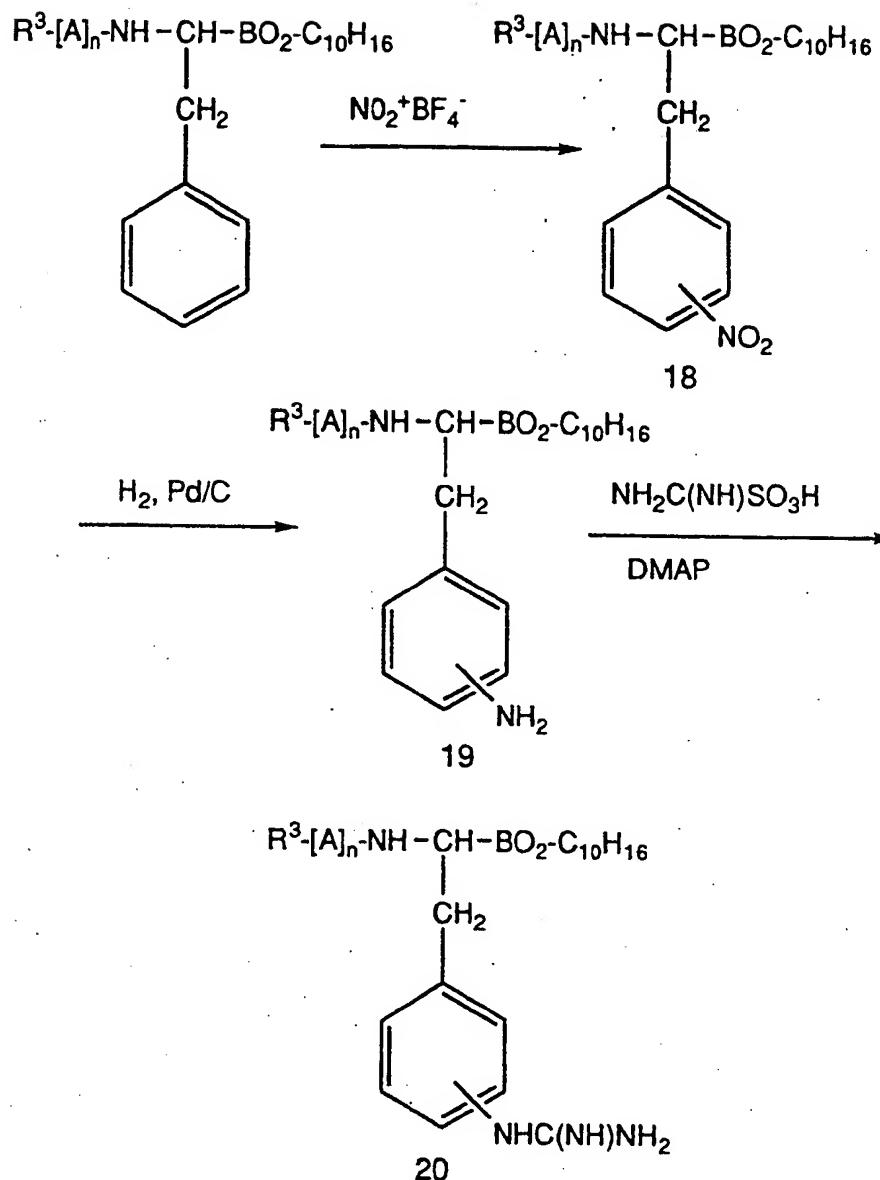
Scheme 6



5

Aromatic guanidino inhibitors, 20, were prepared from precursor R-boroPhe-C10H16, Scheme 7. The aromatic ring was nitrated by reaction with NO^+BF_4^- to yield 18 which was reduced to the corresponding amine, 19. The 10 amine is converted to the guanidine by reaction with aminoiminomethane sulfonic acid [Mosher et al. *Tetrahedral Lett.* **29** 3183 (1988)] or cyanamide (Kettner et al. 1990).

Scheme 7



NMR, proton nuclear magnetic resonance, chemical shifts are reported in δ units, parts per million downfield from the internal tetramethylsilane standard.

5 Elemental analyses were conducted by Galbraith Laboratories Inc., Knoxville, TN and Microanalysis Inc., Wilmington, DE. FAB/MS samples of free boronic acids did not give consistent results making it difficult to

monitor the removal of ester protecting groups by this means. However, the presence of the pinanediol and the pinacol groups are readily observed in NMR spectra. For the pinanediol ester, a methyl group is observed at δ 5 0.9 and the methyl groups of the pinacol groups are observed as singlet at δ 1.1. Following the removal of pinanediol protecting group, MS were run by treating the sample with ~2 equivalents of pinacol in methanol for 5 minutes and evaporating the solvent. Similarly, MS 10 samples of free boronic acid, obtained by removal of the pinacol, were prepared by treating with pinanediol. In some cases, ethylene glycol was used as a matrix for mass spectroscopy to yield the boronic acid-ethyleneglycol ester (designated EG ester). For the 15 subsequent Example see Table 1 for analytical data.

Example 1

Synthesis of Ac-(D)Phe-Pro-NH-CH[(CH₂)₄CN]BO₂-C₁₀H₁₆

The intermediate, Ac-(D)Phe-Pro-NH-CH[(CH₂)₄Br]BO₂-C₁₀H₁₆, was prepared using the mixed anhydride procedure. Ac-(D)Phe-Pro-OH (3.04 g, 10 mmol) was dissolved in 50 mL of THF and N-methylmorpholine (1.1 mL, 10 mmol) was added. The solution was cooled to -20°C using a CCl₄ dry ice bath and isobutyl 25 chloroformate (1.30 mL, 10 mmol) was added. After 5 min at -20°C, the mixture was added to NH₂-CH[(CH₂)₄Br]BO₂-C₁₀H₁₆•HCl (3.81 g, 10 mmol) which was dissolved in 20 mL of THF and precooled to -20°C. Triethylamine (1.39 mL, 10 mmol) was added and the mixture was allowed to stir 30 for 1 h at -20°C and 2 h at room temperature. Insoluble material was removed by filtration and the filtrate was evaporated under a reduced pressure. The residue was dissolved in 50 mL of ethyl acetate and washed subsequently with 75 mL of 0.2 N HCl, 5% NaHCO₃, and 35 saturated aqueous sodium chloride. The organic phase was dried over Na₂SO₄ and concentrated *in vacuo* to give

Ac-(D)Phe-Pro-NHCH[(CH₂)₄Br]BO₂-C₁₀H₁₆ (6.01 g, 95% yield).

The bromide (1.89 g, 3.0 mmol) and tetra-n-butyl ammonium cyanide (3.2 g, 11.8 mmol, 4 eq) were dissolved in 50 mL of acetonitrile. This solution was heated at 90°C for 3 h and solvent was removed under reduced pressure. The residue was dissolved in 50 mL of ethyl acetate and was washed with three 50 mL portions of saturated aqueous NaCl. The ethyl acetate solution was dried over anhydrous Na₂SO₄ and evaporated to give 2.5 g of crude product. It was purified by silica gel chromatography using 5% MeOH in CHCl₃ as an eluent to yield the desired product (0.50 g, 29% yield).

LRMS (NH₃-CI) m/e calcd. for M (C₃₂H₄₅N₄O₅B) + NH₄⁺: 15 594.4. Found: 594. HRMS (NH₃-CI) m/e calcd. for M (C₃₂H₄₅N₄O₅B) + H⁺: 577.3561. Found: 577.3555.

Example 2

Synthesis of Ac-(D)Phe-Pro-NHCH[(CH₂)₄C(NH)NH₂]-BO₂-C₁₀H₁₆·benzene sulfonic acid

The nitrile, (Example 1, 0.40 g, 0.70 mmol), was dissolved in 50 mL of a cold solution of saturated HCl in methanol and the solution was stirred overnight at 4°C. The solution was then concentrated under reduced pressure. The residue was dissolved in anhydrous methanol (50 mL), gaseous NH₃ was bubbled through the solution for 1 h, and the solution was heated at 50 °C for 3 h. Solvent was evaporated, the residue was suspended in minimum volume of methanol, and 0.11 g of benzenesulfonic acid (1 eq) was added. Methanol was evaporated and the residue was triturated with hexane to yield the desired product as a pale yellow powder (0.52 g, 99 % yield).

FABMS: m/e calculated for M (C₃₂H₄₈N₅O₅B) + H⁺: 35 594.38. Found: 594.14. HRMS (NH₃-CI) m/e calcd for M (C₃₂H₄₈N₅O₅B) + H⁺: 594.3827. Found: 594.3824.

Example 3

Synthesis of Ac-(D)Phe-Pro-NHCH[(CH₂)₃NHC(NH)H]BO₂-C₁₀H₁₆
or Ac-(D)Phe-Pro-boroOrn(CH=NH)-C₁₀H₁₆

5 Ethyl formimidate-HCl was prepared by the procedure
of Ohme and Schmitz *Angew. Chem. Internat. Edit.* 6 566
(1967) and Ac-(D)Phe-Pro-boroOrn-C₁₀H₁₆ was prepared by
the procedure of Kettner et al. (1990). The formimidate
(1.29 g, 11.7 mmol) and 4-N,N-dimethylaminopyridine
10 (1.44 g) were added to a solution of Ac-(D)Phe-Pro-
boroOrn-C₁₀H₁₆·BSA (2.78 g, 3.92 mmol) dissolved in 40 mL
of ethanol. The resulting solution was refluxed for 8
h. After removal of solvent, the residue was purified
by chromatography using a column of SephadexTMLH 20 and
15 methanol as a solvent to give pure product (1.28 g, 56 %
yield).

HRMS (NH₃-CI) m/e calcd. for M (C₃₁H₄₆BN₅O₅) + H⁺:
580.3670. Found: 580.3679.

20

Example 4

Synthesis of Ac-(D)Phe-Pro-NHCH[(CH₂)₃-NHC(NH)H]B(OH)₂

The pinanediol protecting group on the boronic acid
portion of Ac-(D)Phe-Pro-NHCH[(CH₂)₃-NHC(NH)H]-BO₂-
C₁₀H₁₆·HCl (Example 3) was removed by

25 transesterification using the procedure we have
described previously in U.S. Application 08/010731. The
pinanediol ester (0.30 g, 0.51 mmol) and phenyl boronic
acid (0.31 g, 2.6 mmol) were suspended in 10 mL of a 1:
1 mixture of ether and water and was allowed to stir for
30 2.5 h at room temperature. The phases were separated
and the aqueous phase was extensively washed with ether.
The aqueous phase was evaporated to yield a solid. This
material was triturated with ether to give the desired
product as an amorphous white solid, 0.20 g (83 %
35 yield). LRMS (NH₃-CI) m/e calcd. for the pinacol ester
M (C₂₇H₄₂N₅O₅B) + H⁺: 528.3. Found: 528. HRMS (NH₃-

CI) m/e calcd. for the pinacol ester M (C₂₇H₄₂N₅O₅B) + H⁺: 528.3357. Found: 528.3347.

Example 5

5 Synthesis of Boc-Pro-NHCH[(CH₂)₃NHC(NH)H]BO₂-C₁₀H₁₆

Boc-Pro-boroOrn-C₁₀H₁₆•BSA was also prepared by the procedure described previously (Kettner et al. 1990).

This peptide (3.0 g, 6.5 mmol) was dissolved in 25 mL of absolute ethanol, 4-N,N-dimethylaminopyridine (1.6 g, 12.9 mmol) and ethyl formimidate•HCl (1.4 g, 12.9 mmol)

10 were added. The solution was heated on a 85 °C oil bath for 1 h. Solvent was evaporated and the residue was dissolved in methanol and was chromatogramed on a 2.5 X 100 cm column of LH20 in methanol to yield 1.3 g of the 15 desired product.

LRMS (NH₃-CI) m/e calcd. for M (C₂₅H₄₃N₄O₅B) + H⁺: 491.5. Found: 491.

Example 6

20 Synthesis of Boc-(D)Phe-Pro-NHCH[(CH₂)₃-NHC(NH)H]BO₂-C₁₀H₁₆

The reaction was run using the procedure described for Example 3. Boc-(D)Phe-Pro-boroOrn-C₁₀H₁₆•BSA (3.7 g, 4.78 mmol), 4-N,N-dimethylaminopyridine (1.71 g, 13.8

25 mmol), and ethyl formimidate•HCl (1.54 g, 13.8 mmol) were dissolved in 50 mL of absolute ethanol and was heated at 85 °C for 7 h. The desired product was obtained by chromatography on a column of LH 20 in a yield of 1.56 g.

30 HRMS (NH₃-CI) m/e calcd for M (C₃₄H₅₂N₅O₆B) + H⁺: 638.4089. Found: 638.4082.

Example 7

35 Synthesis of Boc-(D)Phe-Pro-NHCH[(CH₂)₃-NHC(NH)H]BO₂-C₁₀H₁₆ • 0.40 BSA, 0.60 HCl (Example 6, 0.16

g, 0.22 mmol) and phenyl boronic acid (0.13g, 1.1 mmol) were placed in mixture of 5 mL of ether and 5 mL of water and was allowed to stir for 4 h at room

temperature. The phases were separated and the organic

5 phase was washed with 5 mL of water. The combined aqueous phases were extensively washed with ether. The aqueous phase was evaporated and the residue triturated with ether to yield the desired product as a white solid, 0.10 g. LRMS (NH₃-CI) m/e calcd. for the pinacol ester M (C₃₀H₄₈N₅O₆B) + H⁺: 586.4. Found: 586. HRMS (NH₃-CI) m/e calcd. for the pinacol ester M (C₃₀H₄₈N₅O₆B) + H⁺: 586.3776. Found: 586.3772.

Example 8

15 Synthesis of H-(D)Phe-Pro-NHCH[(CH₂)₃-NHC(NH)H]BO₂-C₁₀H₁₆•2HCl

Boc-(D)Phe-Pro-NHCH[(CH₂)₃-NHC(NH)H]BO₂-C₁₀H₁₆•0.40 BSA, 0.60 HCl (Example 6, 0.20 g, 0.25 mmol) was dissolved in 2 mL of 4 N HCl: dioxane and was allowed to

20 stir for 1 h at room temperature. Solvent was evaporated and the residue was triturated with ether to yield 0.18 g of the desired product.

HRMS (NH₃-CI) m/e calcd for M (C₂₉H₄₄N₅O₄B) + H⁺: 538.3565. Found: 538.3569.

25

Example 9

Synthesis of H-(D)Phe-Pro-NHCH[(CH₂)₃-NHC(NH)H]B(OH)₂

H-(D)Phe-Pro-NH-CH[(CH₂)₃-NH-C(NH)H]BO₂-C₁₀H₁₆•0.35

BSA, 0.65 HCl (Example 8, 0.10 g, 0.16 mmol) was allowed

30 to react with phenyl boronic acid according to the procedure in Example 4 to yield the desired product, 0.053 g. LRMS (NH₃-CI) m/e calcd. for the pinacol ester M (C₂₅H₄₀N₅O₄B) + H⁺: 486.3. Found: 486. HRMS (NH₃-CI) m/e calcd for pinacol ester M (C₂₅H₄₀N₅O₄B) + H⁺:

35 486.3251. Found: 486.3255.

Example 10

Synthesis of H₂NCH[CH₂C₆H₄-*m*-CN]BO₂C₁₀H₁₆·HCl or
H-boroPhe(*m*-CN)-C₁₀H₁₆·HCl

The first intermediate, Cl-CH[CH₂-(*m*-cyanophenyl)]BO₂-C₁₀H₁₆, was prepared from *m*-cyanobenzyl bromide and dichloromethyl boronate pinanediol. Zinc dust (1.0 g) in 1 mL of THF was cooled to 0-5°C and a solution of *m*-cyanobenzyl bromide (1.37 g, 7.0 mmol) in 7 mL of THF was added dropwise (5 sec/drop). The reaction mixture was allowed to stir at 5°C for 2 h. A mixture consisting of LiBr (1.22 g, 14 mmol), CuCN (0.63 g, 7.0 mmol), and 6 mL of THF was placed in a 50 mL flask and cooled to -40°C; then the benzylic organozinc reagent was added by cannulation. The mixture was allowed to warm to -20°C and stir for 5 min. It was cooled to -78°C and neat dichloromethyl boronic acid pinanediol (1.47 g, 5.6 mmol) was added dropwise. The resulting mixture was stirred at -78°C for 2 h and at room temperature for 2 days. Saturated aqueous NH₄Cl (20 mL) was added to the mixture and the aqueous solution was extracted with three 20 mL portions of ether. The combined organic layers was dried over anhydrous MgSO₄ and evaporated *in vacuo* to give crude compound (1.8 g). It was purified by silica gel chromatography where the column was stepwise eluted with hexane (100 mL) and then 15% ether in hexane (200 mL) to give the desired product 0.53 g (27% yield). LRMS (NH₃-CI) m/e calcd. for M (C₁₉H₂₃NO₂BCl)⁺ NH₄⁺: 361.2. Found: 361.1.

To a solution of hexamethyldisilazane (0.21 mL, 0.98 mmol) in 2 mL of THF at -78°C was added *n*-butyllithium (1.45 M, 0.67 mL, 0.98 mmol). The solution was allowed to slowly warm to room temperature to ensure the anion generation was complete. The resulting solution was then cooled to -78°C and Cl-CH[CH₂-(*m*-cyanophenyl)]BO₂-C₁₀H₁₆ (0.33 g, 0.98 mmol) in 2 mL of

THF was added. The mixture was allowed to warm to room temperature and to stir overnight. Solvent was evaporated and 8 mL of hexane was added to give a suspension. HCl in dioxane (4.1 N, 1.5 mL, 6.0 mmol)

5 was added at -78°C. The mixture was slowly warmed to room temperature and stirred for 2 h. Additional hexane (6 mL) was added and crude product was isolated as a precipitate. This product was dissolved in chloroform and insoluble material was removed by filtration. The 10 filtrate was evaporated at a reduced pressure to give an oil (~0.2 g). Final purification was achieved by chromatography on a column of Sephadex™ LH 20 column using methanol as a solvent. H-boroPhe(*m*-CN)-C₁₀H₁₆•HCl was obtained as an oil (0.12 g, 34% yield). HRMS(NH₃-CI) m/e calcd. for M (C₁₉H₂₆BN₂O₂) + H⁺: 325.2087. 15 Found: 325.2094.

Example 11

Synthesis of Ac-(D)Phe-Pro-boroPhe(*m*-CN)-C₁₀H₁₆

20 Ac-(D)Phe-Pro-OH (0.10 g, 0.33 mmol) and N-methylmorpholine (0.037 mL, 0.33 mmol) were allowed to react with isobutyl chloroformate (0.043 mL, 0.33 mmol) in 5 mL of THF at -20°C. After 5 min, H-boroPhe(*m*-CN)-C₁₀H₁₆•HCl, (Example 10, 0.12 g, 0.33 mmol) dissolved in 25 3 mL of cold THF and triethylamine (0.046 mL, 0.33 mmol) were added. The mixture was allowed to stir at -20°C for 1 h and to stir at room temperature for an additional hour. Insoluble material was removed by filtration and solvent was evaporated. The residue was 30 dissolved in ethyl acetate and was washed with 0.20 N HCl, 5 % NaHCO₃, and saturated aqueous NaCl. The organic layer was dried over anhydrous Na₂SO₄ and was evaporated *in vacuo* to give 0.2 g of an oil. It was purified by chromatography on a column of Sephadex™ LH 35 20 yielding 0.13 g of desired product (65% yield).

HRMS (NH₃-CI) m/e calcd. for M (C₃₅H₄₃BN₄O₅) + H⁺:
611.3405. Found: 611.3416.

Example 12

5 Synthesis of Ac-(D)Phe-Pro-boroPhe[m-C(NH)NH₂]-C₁₀H₁₆

Ac-(D)Phe-Pro-boroPhe(*m*-CN)-C₁₀H₁₆, Example 11, (50 mg) was dissolved in 5 mL of saturated solution of HCl in methanol. The solution was allowed to stir overnight at 4 °C. After removal of solvent, the residue was 10 resuspended in 5 mL of anhydrous methanol, cooled to 0°C, and anhydrous NH₃ was bubbled through the solution for 0.5 h. It was heated at 60°C for 6.2 h. Solvent was evaporated and one equivalent of benzene sulfonic acid (13 mg) and 1 mL of methanol were added. Solvent 15 was evaporated under N₂ and the product was triturated with ether to give the desired product as a pale brown powder (65 mg, 100% yield). HRMS (NH₃-CI) m/e calcd. for M (C₃₅H₄₇BN₅O₅) + H⁺: 628.3670. Found: 628.3688.

20

Example 13

Synthesis of Ac-(D)Phe-Pro-boroPhe(*m*-CH₂NH₂)-C₁₀H₁₆

Ac-(D)Phe-Pro-boroPhe(*m*-CN)-C₁₀H₁₆ was placed in 5 mL of methanol, 10% Pd/C (25 mg) and 0.1N HCl (0.41 mL) were added, and the mixture was stir under H₂ at room 25 temperature for 2.5 h. The solution was filtered through Celite and washed with 20 mL of methanol. The filtrate was concentrated under a reduced pressure and the residue was triturated with ether to give pure product as white powder (15.6 mg, 59% yield). HRMS (NH₃-CI) m/e calcd. for M (C₃₅H₄₇N₄O₅B) + H⁺: 615.3718. Found: 615.3700.

Example 14

Synthesis of Ac-(D)Phe-Pro-boroPhe(*m*-Br)-C₁₀H₁₆

35 Cl-CH[CH₂-(*m*-bromo-phenyl)]BO₂-C₁₀H₁₆ was prepared making the anion of *m*-bromobenzyl bromide and coupling

it to dichloromethyl boronic acid pinanediol. This intermediate and the corresponding amine were prepared using the procedure described for Example 10. The amine was coupled to Ac-(D)Phe-Pro-OH using the method

5 described in Example 11.

LRMS(NH₃-CI) m/e calcd. for M (C₃₄H₄₃N₃O₅BrB) + H⁺:
666.3. Found: 666.2.

Example 15

10 Synthesis of Ac-(D)Phe-Pro-boroArg(CN)-C₁₀H₁₆

Ac-(D)Phe-Pro-boroOrn-C₁₀H₁₆·HCl (0.15 g, 0.25 mmol), triethylamine (0.035 mL, 0.25 mmol), and diphenyl cyanocarbonimidate (Aldrich, 0.060 g, 0.25 mmol) were heated at a gentle reflux for 5 h in THF and then 15 stirred overnight at room temperature. The sample was diluted with chloroform and washed with water and saturated aqueous NaCl. It was dried over K₂CO₃ and purified by silica gel chromatography using methanol: chloroform (1:9) as a solvent to yield 80 mg of Ac-(D)Phe-Pro-NH-CH[(CH₂)₃-NH-C(N-CN)O-Ph]BO₂-C₁₀H₁₆.
20 LRMS(NH₃-CI) m/e calcd. for M (C₃₈H₄₉N₆O₆B) + H⁺: 697.7. Found: 697.

The above product (0.060 g, 0.080 mmol) was dissolved in 0.5 mL of THF and was allowed to react with

25 1 equivalent of 30% aqueous ammonia for 30 min at room temperature. Four additional equivalent of ammonia were added and the solution was allowed to stir overnight at room temperature. A large excess of ammonia was added and the reaction mixture was allowed to stir 2 days at 30 room temperature. The reaction mixture was diluted with methylene chloride and was washed with water and saturated aqueous NaCl. It was dried over K₂CO₃ and purified by chromatography on a silica gel column using methanol and chloroform (1:9) as a solvent to yield 15 35 mg of the desired product. LRMS(NH₃-CI) m/e calcd. for M (C₃₂H₄₆N₇O₅B) + H⁺: 619.5. Found: 620.

Example 16

Synthesis of Ac-(D)Phe-Pho-boroPhe(p-CN)-C₁₀H₁₆

ClCH[CH₂C₆H₄-p-CN]BO₂C₁₀H₁₆ was prepared by making
5 the anion of *p*-cyanobenzyl bromide and coupling it to
dichloromethyl boronate pinanediol. This intermediate
and the corresponding amine were prepared using the
procedure described for Example 10. NH₂CH[CH₂C₆H₄-p-
CN]BO₂C₁₀H₁₆ (Example 78) was coupled to Ac-(D)Phe-Pro-
10 OH using the method described in Example 11.

HRMS (NH₃-Cl)^{m/e} calcd. for M (C₃₅H₄₃N₄O₅B) + H⁺:
611.3405. Found: 611.3408.

Example 17

15 Synthesis of Boc-(D)Phe-Pro-boroPhe(mCN)-C₁₀H₁₆

Boc-(D)Phe-Pro-boroPhe(mCN)-C₁₀H₁₆ was prepared by
reacting Boc-(D)Phe-Pro-OH (0.43 g, 1.2 mmol), H-
boroPhe(mCN)-C₁₀H₁₆•HCl (0.42 g, 1.2 mmol), N-
methylmorpholine (0.26 mL, 2.4 mmol),
20 hydroxybenzotriazole•H₂O (0.36 g, 2.4 mmol), and
dicyclohexylcarbodiimide (0.25 g, 1.2 mmol) in 20 mL of
dichloromethane overnight at room temperature. The
reaction mixture was filtered and the filtrate was
chromatographed on a 2.5 X 100 cm column of Sephadex LH-
25 20 in methanol to yield 0.36 g of the desired product.

Example 18

Synthesis of H-(D)Phe-Pro-boroPhe(mCN)-C₁₀H₁₆•HCl

Boc-(D)Phe-Pro-boroPhe(mCN)-C₁₀H₁₆ (0.21 g) was
30 allowed to react with 2 mL of 4 N HCl dioxane for 2 h at
room temperature. Solvent was removed by evaporation
and the residue was triturated with ether to yield 0.11
g of the desired product as a white solid.

35

Example 19

Synthesis of H-(D)Phe-Pro-boroPhe(mCN)-OH•HCl

H-(D)Phe-Pro-boroPhe(mCN)-C₁₀H₁₆·HCl (0.63 g, 1.0 mmol) was allowed to react with 5 equivalents of phenylboronic acid using the procedure described for Example 7 to yield 0.46 g of product.

5

Example 20

Synthesis of N,N Dimethyl-(D)Phe-Pro-boroPhe(mCN)-OH·HCl

H-(D)Phe-Pro-boroPhe(mCN)-OH·HCl (0.20 g, 0.42 mmol), 37% aqueous formaldehyde (0.34 mL, 4.2 mmol) were dissolved in 2 mL of acetonitrile. Sodium cyanoborohydride (0.080 g, 1.3 mmol) was added and after 5 min glacial acetic acid (20 µL) were added. The reaction pH was ~7. After 5 h, additional acetic acid (20 µL) were added and the mixture was stirred for 1 h. The reaction mixture was poured into 20 mL of ethyl acetate and the organic phase was washed with 10 mL of saturated aqueous sodium chloride and dried over anhydrous sodium sulfate. Evaporation of solvent yielded 0.16 g of an oil which was triturated with ether to give a white solid.

Example 52

Synthesis of Ac-(D)Phe-Pro-NH-H[(CH₂)₃SC(NH)NHCH₃]B(OH)₂

The intermediate, Ac-(D)Phe-Pro-NH-CH[(CH₂)₃Br]BO₂C₁₀H₁₆, was prepared using the mixed anhydride procedure of example 1. A solution of this bromide (0.35 g, 0.57 mmol) and 1-methyl-2-thiourea (0.077 g, 0.85 mmol) in 10 mL of absolute ethanol was refluxed for 18 hours. After cooling the solvent was removed under vacuum, and the product was separated from excess thiourea employing chromatography (elution: methanol) on Sephadex® LH-20 gel to provide 0.31 g (77%) of the isothiouronium product. This boronic acid ester (0.28 g) was then deprotected as described in example 4 to afford 0.13 g (57%) of the desired product. LRMS (ESI) m/e calcd. for M (C₂₂H₃₄BN₅O₅S) + H⁺: 492. Found:

492. HRMS (NH₃-CI) m/e calcd. for ethylene glycol ester M (C₂₄H₃₆BN₅O₅S) + H⁺: 518.260847. Found: 518.261656.

Example 54

5 Synthesis of Ac-(D)Phe-Pro-NH-CH₁(CH₂)₃NHC(NH)NHCH₃1-B(OH)₂

A solution of Ac-(D)Phe-Pro-boroOrn-BO₂C₁₀H₁₆ • HCl [0.50 g, 0.85 mmol, prepared by the procedure of Kettner et al. (1990)], 4-methylaminopyridine (0.21 g, 1.7 mmol), 10 N-methylamino-iminomethanesulfonic acid (0.24 g, 1.7 mmol), and 10 mL of absolute ethanol was refluxed for 18 hours. After cooling the mixture was filtered and the precipitate was washed with chloroform. The combined filtrates were concentrated under vacuum, and the 15 residue was dissolved in 10 mL of chloroform. The chloroform solution was washed with ice-cold 0.1 N hydrochloric acid (2 X 3 mL), ice-cold water (2 X 3 mL), and brine. The resulting organic solution was then dried over anhydrous magnesium sulfate, filtered, and 20 concentrated. The product was purified employing chromatography (elution: methanol) on Sephadex[®] LH-20 gel to provide 0.30 g (55%) of the guanidine. This boronic acid ester was then deprotected as described in example 4 to afford 0.14 g (59%) of the desired product. 25 LRMS (NH₃-CI) m/e calcd. for ethylene glycol ester M (C₂₄H₃₇BN₆O₅) + H⁺: 501. Found: 501. HRMS (NH₃-CI) m/e calcd. for ethylene glycol ester M (C₂₄H₃₇BN₆O₅) + H⁺: 501.299674. Found: 501.300760.

30 The examples of Table 1 can be prepared by the schemes and procedures described above using the appropriate starting materials.

Table 1.

EX #	Compound	MS Method	LRMS CALC'D	LRMS FOUND
1	Ac-(D)Phe-Pro-NH-CH[(CH ₂) ₄ CN]BO ₂ C ₁₀ H ₁₆	NH ₃ /Cl (M+NH ₄)	594.4	594
2	Ac-(D)Phe-Pro-NH-CH[(CH ₂) ₄ -C(NH)NH ₂]BO ₂ C ₁₀ H ₁₆ ·BSA	NH ₃ /Cl (M+H)	594.4	594
3	Ac-(D)Phe-Pro-boroOrn(CH=NH)]-C ₁₀ H ₁₆ ·HCl	NH ₃ /Cl (M+H)	580.4	580
4	Ac-(D)Phe-Pro-boroOrn(CH=NH)]-OH·HCl	NH ₃ /Cl pinacol ester+H	528.3	528
5	Boc-Pro-boroOrn(CH=NH)-C ₁₀ H ₁₆ ·HCl	NH ₃ /Cl (M+H)	491.5	491
6	Boc-(D)Phe-Pro-boroOrn(CH=NH)]-C ₁₀ H ₁₆ ·0.5 HCl·0.5 BSA	NH ₃ /Cl (M+H)	638.4	638
7	Boc-(D)Phe-Pro-boroOrn(CH=NH)]-OH·0.6 HCl·0.4 BSA	NH ₃ /Cl pinacol ester+H	586.4	586
8	H-(D)Phe-Pro-boroOrn(CH=NH)]-C ₁₀ H ₁₆ ·0.5 HCl·0.5 BSA	NH ₃ /Cl (M+H)	538.4	538
9	H-(D)Phe-Pro-boroOrn(CH=NH)]-OH·0.65 HCl·0.35 BSA	NH ₃ /Cl pinacol ester+H	486.3	486
10	H-boroPhe(mCN)-C ₁₀ H ₁₆ ·HCl			
11	Ac-(D)Phe-Pro-boroPhe-(m-CN)-C ₁₀ H ₁₆	NH ₃ /Cl (M+H)	611.3	611
12	Ac-(D)Phe-Pro-boroPhe-(m-C(NH)NH ₂)-C ₁₀ H ₁₆ ·BSA	NH ₃ /Cl (M+H)	628.4	628
13	Ac-(D)Phe-Pro-boroPhe-(m-CH ₂ NH ₂)-C ₁₀ H ₁₆ ·HCl	NH ₃ /Cl (M+H)	615.4	615
14	Ac-(D)Phe-Pro-boroPhe-(m-Br)-C ₁₀ H ₁₆	NH ₃ /Cl (M+NH ₄)	683.4	683
15	Ac-(D)Phe-Pro-boroArg(CN)-C ₁₀ H ₁₆ ·HCl	NH ₃ /Cl (M+H)	619.5	620
16	Ac-(D)Phe-Pro-boroPhe-(p-CN)-C ₁₀ H ₁₆	NH ₃ /Cl (M+NH ₄)	628.4	628
17	Boc-(D)Phe-Pro-boroPhe-(m-CN)-C ₁₀ H ₁₆	NH ₃ /Cl (M+NH ₄)	686.4	686

18	H-(D)Phe-Pro-boroPhe-(m-CN)-C ₁₀ H ₁₆ •HCl	NH ₃ /Cl (M+H)	569.3	569
19	H-(D)Phe-Pro-boroPhe-(m-CN)-OH•HCl	NH ₃ /Cl	461.2	461
20	N,N-(CH ₃) ₂ -(D)Phe-Pro-boroPhe-(m-CN)-OH•HCl (ISOMER I)	EG ester+H NH ₃ /Cl EG ester+H	489.3	489
21	Ac-(D)Phe-Pro-boroPhe-(p-CH ₂ NH ₂)-C ₁₀ H ₁₆ • BSA	NH ₃ /Cl (M+H)	615.4	615
22	Ac-(D)Phe-Pro-boroPhe-(p-C(NH)NH ₂)-C ₁₀ H ₁₆ • BSA	FAB (M+H)	628.37	628.44
23	Ac-(D)Phe-Pro-boroPhe-(m-CN)-OH•HCl	NH ₃ /Cl EG ester+NH ₄	520.3	520
24	Ms-(D)Phe-Pro-boroPhe-(m-CN)-OH•HCl	NH ₃ /Cl EG ester+NH ₄	556.2	556
25	N-CH ₃ -(D)Phe-Pro-boroPhe-(m-CN)-C ₁₀ H ₁₆ •HCl	NH ₃ /Cl (M+H)	583.4	583.3
26	H-Pro-boroPhe-(m-CN)-C ₁₀ H ₁₆ •HCl	NH ₃ /Cl (M+H)	422.3	422
27	Boc-(D)Thiazolylalanine-Pro-boroPhe-(m-CN)-C ₁₀ H ₁₆	NH ₃ /Cl (M+H)	676.4	676.4
28	Boc-(D)3-Pyridylalanine-Pro-boroPhe-(m-CN)-C ₁₀ H ₁₆	NH ₃ /Cl (M+H)	670.4	670.4
29	H-(D)Thiazolylalanine-Pro-boroPhe-(m-CN)-C ₁₀ H ₁₆ •HCl	NH ₃ /Cl (M+H)	576.3	576
30	H-(D)3-Pyridylalanine-Pro-boroPhe-(m-CN)-C ₁₀ H ₁₆ •HCl	NH ₃ /Cl (M+H)	570.3	570
31	Ms-(D)Thiazolylalanine-Pro-boroPhe-(m-CN)-C ₁₀ H ₁₆	NH ₃ /Cl (M+H)	654.3	654
32	Ms-(D)3-Pyridylalanine-Pro-boroPhe-(m-CN)-C ₁₀ H ₁₆	NH ₃ /Cl (M+H)	648.3	648
33	N-Boc-N-CH ₃ -(D)Phe-Pro-boroPhe-(m-CN)-C ₁₀ H ₁₆	NH ₃ /Cl (M+NH ₄)	700.4	700

34	Boc-(D)2-Pyridylalanine-Pro-boroPhe-(m-CN)-C ₁₀ H ₁₆	NH ₃ /Cl (M+H)	670.4	670
35	Ac-Pro-boroPhe-(m-CN)-C ₁₀ H ₁₆	NH ₃ /Cl (M+NH4)	481.3	481
36	Boc-(D)2-Thienylalanine-Pro-boroPhe-(m-CN)-C ₁₀ H ₁₆	NH ₃ /Cl (M+NH4)	692.4	692
37	H-(D)2-Pyridylalanine-Pro-boroPhe-(m-CN)-C ₁₀ H ₁₆ •HCl	NH ₃ /Cl (M+H)	570.3	570
38	H-(D)2-Thienylalanine-Pro-boroPhe-(m-CN)-C ₁₀ H ₁₆ •HCl	NH ₃ /Cl (M+H)	575.3	575
39	Ms-(D)2-Pyridylalanine-Pro-boroPhe-(m-CN)-C ₁₀ H ₁₆	NH ₃ /Cl (M+H)	648.3	648
40	Ms-(D)2-Thienylalanine-Pro-boroPhe-(m-CN)-C ₁₀ H ₁₆	NH ₃ /Cl (M+NH4)	670.3	670
41	(2-Pyrimidylthio)acetyl-Pro-boroPhe-(m-CN)-C ₁₀ H ₁₆	NH ₃ /Cl (M+H)	574.3	574
42	trans-3-(3-pyridyl)acryl-Pro-boroPhe-(m-CN)-C ₁₀ H ₁₆	NH ₃ /Cl (M+H)	553.3	553
43	(4-Pyridylthio)acetyl-Pro-boroPhe-(m-CN)-C ₁₀ H ₁₆	NH ₃ /Cl (M+H)	573.3	573
44	Succinyl-(D)Phe-Pro-boroPhe-(m-CN)-OH	NH ₃ /Cl EG ester+NH4	578.3	578
45	3-Pyridylpropionyl-Pro-boroPhe-(m-CN)-C ₁₀ H ₁₆	NH ₃ /Cl (M+H)	553.3	555
46	Boc-(D)Phe-Aze-boroPhe-(m-CN)-C ₁₀ H ₁₆	NH ₃ /Cl (M+NH4)	672.4	672
47	H-(D)Phe-Aze-boroPhe-(m-CN)-C ₁₀ H ₁₆ •HCl	NH ₃ /Cl (M+H)	555.3	555
48	Hydrocinnamoyl-Pro-boroOrn(CH=NH)OH•BSA	FAB EG ester+H	445.5	445
49	Hydrocinnamoyl-Pro-boroIrg(CH ₂ CH=CH ₂)-OH • HBr	ESI (M+H)	461	461
50	Hydrocinnamoyl-Pro-boroIrg(CH ₃)-OH • HBr	ESI (M+H)	435	435

51	Cbz-(D)Phe-Pro-boroIrg(CH ₃)-C ₁₀ H ₁₆ • HBr	NH ₃ /Cl (M+H)	718	718
52	Ac-(D)Phe-Pro-boroIrg(CH ₃)-OH • HBr	ESI (M+H)	492	492
53	Hydrocinnamoyl-Pro-boroIrg(CH ₂ CH ₃)-OH • HBr	ESI (M+H)	449	449
54	Ac-(D)Phe-Pro-boroArg(CH ₃)-OH • HCl	NH ₃ /Cl EG ester+H	501	501
55	Hydrocinnamoyl-Pro-boroArg(CH ₃)-OH • HCl	ESI (M+H)	418	418
56	Ms-(D)Phe-Pro-boroArg(CH ₃)-OH • HCl	ESI (M+H)	511	511
57	Ms-(D)Phe-Pro-boroOrn(CH=NH)-OH • HCl	ESI (M+H)	482	482
58	PhSO ₂ -(D)Phe-Pro-boroArg(CH ₃)-OH • HCl	ESI (M+H)	573	573
59	PhSO ₂ -(D)Phe-Pro-boroOrn(CH=NH)-OH • HCl	ESI (M+H)	544	544
60	Ms-(D)Phe(4-fluoro)-Pro-boroOrn(CH=NH)-OH • HCl	ESI (M+H)	500	500
61	PhCH ₂ SO ₂ -(D)Phe-Pro-boroArg(CH ₃)-OH • HCl	ESI (M+H)	587	587
62	PhCH ₂ SO ₂ -(D)Phe-Pro-boroOrn(CH=NH)-OH • HCl	ESI (M+H)	558	558
63	CH ₃ CH ₂ CH ₂ SO ₂ -(D)Phe-Pro-boroOrn(CH=NH)-OH • HCl	ESI (M+H)	510	510
64	CH ₃ CH ₂ CH ₂ SO ₂ -(D)Phe-Pro-boroArg(CH ₃)-OH • HCl	ESI (M+H)	539	539
65	CH ₃ (CH ₂) ₃ SO ₂ -(D)Phe-Pro-boroArg(CH ₃)-OH • HCl	ESI (M+H)	553	553
66	CH ₃ (CH ₂) ₃ SO ₂ -(D)Phe-Pro-boroOrn(CH=NH)-OH • HCl	ESI (M+H)	524	524
67	Ac-(D)Phe-Sar-boroOrn(CH=NH)-OH • HCl			
68	Ms-(D)Phe-Sar-boroOrn(CH=NH)-OH • H			

69	Phenethyl-SO ₂ -(D)Phe-Sar-boroOrn(CH=NH)-OH•HCl		
70	Boc-(D)Phe-Sar-boroOrn(CH=NH)-OH•HCl		
71	N-alpha-[boroOrn(CH=NH)-OH]-(2-trans benzylcarboxamido)-cyclopentane-1- carboxamide•HCl		
72	H-(D)Phe-Sar-boroOrn(CH=NH)- C ₁₀ H ₁₆ •2HCl		
73	Boc-(D)Phe-Sar-boroPhe(m-CN)-C ₁₀ H ₁₆		
74	Boc-(D)Phe-Aze-boroOrn(CH=NH)- OH•HCl		
75	H-(D)Phe-Sar-boroPhe(m-CN)- C ₁₀ H ₁₆ •2HCl		
76	4-(Phenyl)benzoyl-boroOrn(CH=NH)- C ₁₀ H ₁₆ •HCl		
77	Z-(D)Phe-Pro-boroOrn(CH=NH)-OH•HCl	NH ₃ /Cl pinacol ester+H	620.58 620.36
78	H-boroPhe-(p-CN)-C ₁₀ H ₁₆ •HCl		
79	Boc-(D)Phe-Pro- N(CH ₃)CH[(CH ₂) ₃ NHC(NH)H]-B(OH) ₂		
80	Boc-(D)Phe-Pro- N(Phenyl)CH[(CH ₂) ₃ NHC(NH)H]-B(OH) ₂		
81	Boc-(D)Phe-Pro- N(benzyl)CH[(CH ₂) ₃ NHC(NH)H]-B(OH) ₂		
82	Boc-(D)Phe-Pro- N(CH ₃)CH[(CH ₂) ₃ NHC(NH)H]-B(OMe) ₂		
83	Boc-(D)Phe-Pro- N(CH ₃)CH[(CH ₂) ₃ NHC(NH)H]-B[N(Me)] ₂		
84	Boc-(D)Phe-Pro- N(CH ₃)CH[(CH ₂) ₃ NHC(NH)H]-B(F) ₂		
85	FMoc-(D)Phe-Pro- NHCH[(CH ₂) ₃ NHC(NH)H]-B(OC ₁₀ H ₁₆) ₂		

86 Ac-(D)cyclohexylalanyl-Pro-
NHCH[(CH₂)₃NHC(NH)H]-B(OC₁₀H₁₆)₂

87 Ac-(D)Phe-Gly-NHCH[(CH₂)₃NHC(NH)H]-
B(OC₁₀H₁₆)₂

88 Ac-(D)Phe-Pro-
NHCH[(CH₂)₃NHC(NOH)NH₂]-
B(OC₁₀H₁₆)₂

91 Ac- (D)Phe-Pro-boroPhe-(p-Br)-C₁₀H₁₆

92 Ac- (D)Phe-Pro-boroPhe-(p-NH₂)-C₁₀H₁₆

93 Ac- (D)Phe-Pro-boroPhe-(p-
NHC(NH)NH₂)-C₁₀H₁₆

95 Ac- (D)Phe-Pro-boroPhe-(p-
CH₂NHC(NH)NH₂)-C₁₀H₁₆

96 Ac- (D)Phe-Pro-boroPhe-(m-
CH₂NHC(NH)NH₂)-C₁₀H₁₆

97 Ac- (D)Phe-Pro-boroPhe-(m-
CH₂NHC(NH)NHCN)-C₁₀H₁₆

98 Z-Leu-Ser(Ot-Bu)-Asn-Leu-Ser(Ot-Bu)-
Asn-Leu-Ser(Ot-Bu)-Asn-Leu-Ser(Ot-Bu)-
Asn-NHCH[(CH₂)₃NHC(NH)H]-
B(OC₁₀H₁₆)₂

99 H-Leu-Ser(Ot-Bu)-Asn-Leu-Ser(Ot-Bu)-
Asn-Leu-Ser(Ot-Bu)-Asn-Leu-Ser(Ot-Bu)-
Asn-NHCH[(CH₂)₃NHC(NH)H]-
B(OC₁₀H₁₆)₂

100 Z-Leu-Ser-Asn-Leu-Ser-Asn-Leu-Ser-Asn-
Leu-Ser-Asn-NHCH[(CH₂)₃NHC(NH)H]-
B(OC₁₀H₁₆)₂

101 H-Leu-Ser-Asn-Leu-Ser-Asn-Leu-Ser-Asn-
Leu-Ser-Asn-NHCH[(CH₂)₃NHC(NH)H]-
B(OC₁₀H₁₆)₂

Utility

5 N-Acyl and N-peptide boronic acids which are described in the present invention represent a novel class of potent, reversible inhibitors of trypsin-like enzymes. Trypsin-like enzymes are a group of proteases which hydrolyzed peptide bonds at basic residues

10 liberating either a C-terminal arginyl or lysyl residue. Among these are enzymes of the blood coagulation and fibrinolytic system required for hemostasis. They are Factors II, X, VII, IX, XII, kallikrein, tissue plasminogen activators, urokinase-like plasminogen

15 activator, and plasmin. Enzymes of the complement system, acrosin (required for fertilization), pancreatic trypsin are also in this group. Elevated levels of proteolysis by these proteases can result in disease states. For example, consumptive coagulopathy, a

20 condition marked by a decrease in the blood levels of enzymes of both the coagulation system, the fibrinolytic system and accompanying protease inhibitors is often fatal. Intervention by a synthetic inhibitor would clearly be valuable. More specifically, proteolysis by

25 thrombin is required for blood clotting. Inhibition of thrombin results in an effective inhibitor of blood clotting. The importance of an effective inhibitor of thrombin is underscored by the observation that conventional anticoagulants such as heparin (and its

30 complex with the protein inhibitor, antithrombin III) are ineffective in blocking arterial thrombosis associated with myocardial infarctions and other clotting disorders. However, a low molecular weight thrombin inhibitor, containing a different

35 functionality, was effective in blocking arterial thrombosis [Hanson and Harker (1988) *Proc. Natl. Acad.*

Sci. U.S.A. 85, 3184-3188]. Therefore, we have chosen to demonstrate utility of compounds in the inhibition of thrombin, both as in buffered solutions and in plasma.

Specifically, the compounds have utility as drugs for

5 the treatment of diseases arising from elevated thrombin activity such as myocardial infarction, and as reagents used as anticoagulants in the processing of blood to plasma for diagnostic and other commercial purposes.

When used in the processing of blood products, the

10 compounds of this invention may be mixed with whole blood without the need for any additional anticoagulants. The compounds of this invention serve to inhibit blood coagulation thereby facilitating the processing of whole blood into desired cellular

15 components or plasma proteins. Once the processing is complete, the compounds may be removed, if so desired, by membrane ultrafiltration, dialysis, or diafiltration to afford the desired product. The low molecular weight of these compounds relative to conventional

20 anticoagulants like heparin allow them to be separated from desired products more easily.

Compounds of the present invention are expected to be effective in the control of aberrant proteolysis and a number of accompanying disease states such as

25 inflammation, pancreatitis, and hereditary angioedema.

The in vitro effectiveness of compounds of the present invention as inhibitors of the blood coagulation protease thrombin was determined under two different conditions: (1) measurements in buffered solutions

30 using a synthetic substrate; (2) measurement in plasma where the rate of blood clotting is determined. For the former, the chromogenic substrate S2238 (H-(D)Phe-Pip-Arg-PNA, where PIP refers to pipecolic acid; Helena Laboratories, Beaumont, TX) was used following

35 procedures similar to those described in Kettner et al.

J. Biol. Chem. 265 18289-18297 (1990). Here hydrolysis

resulted in the release of pNA which was monitored spectrophotometrically by measuring the increase in absorbance at 405 nm. The Michaelis constant, K_m , for substrate hydrolysis was determined at 25 °C in 0.10 M sodium phosphate buffer, pH 7.5, containing 0.20 M NaCl, and 0.5 % PEG 8000 using the method of Lineweaver and Burk.

Values of K_i were determined by allowing thrombin (0.19 nM) to react with substrate (0.20 mM) in the presence of inhibitor. Reactions were allowed to go for 30 minutes and the velocities (rate of absorbance change vs time) were measured in the time frame of 25-30 minutes. The following relationship was used to calculate K_i values.

15

$$\frac{v_0 - v_s}{v_s} = \frac{I}{K_i(1 + S/K_m)}$$

where

20 v_0 is the velocity of the control in the absence of inhibitor;
 v_s is the velocity in the presence of inhibitor;
 I is the concentration of inhibitor;
 K_i is the dissociation constant of enzyme: inhibitor
25 complex;
 S is the concentration of substrate;
 K_m is the Michaelis constant.

Inhibition of blood clotting activity of thrombin in plasma was determined in rabbit plasma. Plasma was 30 prepared by diluting blood 1:10 with 3.2% aqueous citric acid and centrifuging. Buffer consisted of 0.10 M Tris, pH 7.4, containing 0.9% sodium chloride, and 2.5 mg/mL bovine serum albumin. Bovine thrombin was obtained from Sigma and was diluted to 24 NIH units/mL. Plasma (200 35 μ L) and buffer (50 μ L) containing inhibitor were incubated 3 min at 37 °C in a fibrameter. Reactions

were initiated by adding thrombin (50 μ L) and clotting times measured. Controls were run under identical conditions except in the absence of inhibitor. The final concentration of thrombin was 4 NIH units/mL.

5

Table 2 - Inhibition of Thrombin.

Ex #	K _i a (nM)
1	750
2	0.26
3	0.38
4	0.28
6	0.085
7	0.040
8	0.18
9	.05
11	3.2
12	2.8
13	4.83
14	10
15	40
16	134
17	0.27
20	0.14
23	0.55
24	0.059
27	0.17
28	0.37
32	0.48
34	0.33
36	0.381
40	0.19
46	0.55
50	<859
54	1

62	0.03
63	0.5
64	0.5
67	8.2
73	81
74	<0.5
76	110

a Ki values were measured at 25 °C at pH 7.5.

Another measure of compound effectiveness toward prolonging clotting times can be reported as IC50 (level of inhibitor required to prolong clotting to the time observed for 2 NIH units/mL thrombin in the absence of inhibitor). Representative of data for compounds of the present invention, Examples 3, 7, 9, 11, and 12 increased thrombin clotting times 2-fold at 0.25, <0.075, 0.10, 0.60, and 0.85 μM, respectively.

The effectiveness of compounds of the present invention as anticoagulants *in vivo* was demonstrated by the prolongation of the activated partial thromboplastin time of samples of blood taken from conscious dogs or anesthetized rats after either oral or intravenous administration at doses of the compounds from 0.5 to 10 mg/kg. Arterial or venous blood was withdrawn by syringe and mixed with 1/10 volume 3.2% sodium citrate. Plasma was obtained after centrifugation and a standard clinical activated partial thromboplastin time (APTT) reagent, Sigma Chemical Co., St. Louis, Mo.) determined at 37°C in a fibrometer. Results from blood samples obtained at various times after dosing showed an effective anticoagulant response which was at least equivalent to doubling of activated partial thromboplastin time as compared to the value obtained prior to dosing. In this model, Examples 4, 57, and 77

were shown to be effective following i.v. dosing and Examples 4, 56, 57, 60, and 66 effective following oral dosing. Similarly, oral administration of Examples 31 and 54 resulted in at least a 2-fold elevation in 5 anticoagulant activity in an identical model except activity was measured by increases in thrombin clotting times.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Sheng-Lian O. Lee

5

John Matthew Fevig

Charles Adrian Kettner

David L. Carini

(ii) TITLE OF INVENTION: Amidino and Guanidino

10 Substituted Boronic Acid Inhibitors of Trypsin-Like Enzymes

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:

15 (A) ADDRESSEE: The Du Pont Merck Pharmaceutical
Company

(B) STREET: 1007 Market Street, Legal Department

(C) CITY: Wilmington

(D) STATE: DE

20 (E) COUNTRY: U.S.

(F) ZIP: 19898

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.50 inch disk

25 (B) COMPUTER: Apple Macintosh

(C) OPERATING SYSTEM: Apple Macintosh

(D) SOFTWARE: Microsoft Word

(vi) CURRENT APPLICATION DATA:

30 (A) APPLICATION NUMBER: 08/052,835

(B) FILING DATE: _____

(C) CLASSIFICATION: unknown

(vii) PRIOR APPLICATION DATA: None

35

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Reinert, Norbert, F.
- (B) REGISTRATION NUMBER: 18,926
- (C) REFERENCE/DOCKET NUMBER: DM-6567-A

5

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 302-892-8867
- (B) TELEFAX: 302-892-8536

10 (2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12
- (B) TYPE: amino acids
- (C) TOPOLOGY: linear

15 (ii) MOLECULAR TYPE: peptide

(vi) ORIGINAL SOURCE: synthetic

(ix) FEATURE:

(D) OTHER INFORMATION: Example Number 98
at page 36 and within Table 1

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Xaa	Xaa	Asn	Leu	Xaa	Asn	Leu	Xaa	Asn	Leu	Xaa	Asn
1				5						10	

25

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12
- (B) TYPE: amino acids
- (C) TOPOLOGY: linear

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Leu Xaa Asn Leu Xaa Asn Leu Xaa Asn Leu Xaa Asn
1 5 10

5

(3) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULAR TYPE: peptide

(vi) ORIGINAL SOURCE: synthetic

(ix) FEATURE:

(D) OTHER INFORMATION: Example Number 100
15 at page 36 and within Table 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Xaa Ser Asn Leu Ser Asn Leu Ser Asn Leu Ser Asn
20 1 5 10

(3) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULAR TYPE: peptide

(vi) ORIGINAL SOURCE: synthetic

(ix) FEATURE:

(D) OTHER INFORMATION: Example Number 101
30 at page 36 and within Table 1

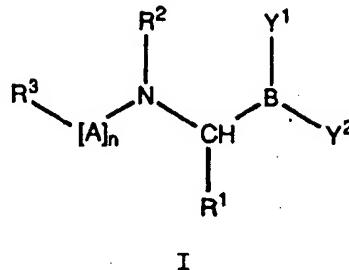
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Leu Ser Asn Leu Ser Asn Leu Ser Asn Leu Ser Asn
35 1 5 10

What is Claimed is:

1. A compound of formula (I)

5

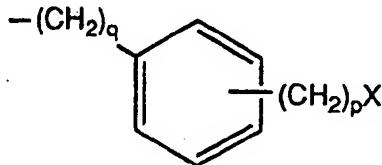


wherein

R¹ is

10 a) C₁-C₁₂-alkyl substituted with -CN, -C(NH)NHR⁶,
 -NHC(NH)H, -NHC(NH)NHR⁶, -SC(NH)NHR⁶, -NHC(NH)NHOH,
 -NHC(NH)NHCN, -NHC(NH)NHCOR⁶, or

b)



X is

15 a) halogen (F, Cl, Br, I)
 b) -CN,
 c) -NO₂,
 d) -CF₃,
 e) -NH₂
 20 f) -NHC(NH)H,
 g) -NHC(NH)NHOH,
 h) -NHC(NH)NHCN,
 i) -NHC(NH)NHR⁶,
 j) -NHC(NH)NHCOR⁶,
 25 k) -C(NH)NHR⁶,
 l) -C(NH)NHCOR⁶,
 m) -C(O)NHR²,
 n) -CO₂R²,

- o) $-\text{OR}^2$, or
- p) $-\text{OCF}_3$
- q) $-\text{SC}(\text{NH})\text{NHR}^6$;

R^2 is

5 a) H,
b) C1-C4-alkyl,
c) aryl, wherein aryl is phenyl or napthyl
optionally substituted with one or two substituents
selected from the group consisting of halo (F, Cl,
10 Br, I), C1-C4-alkyl, C1-C4-alkoxy, $-\text{NO}_2$, $-\text{CF}_3$,

$-\text{S}(\text{O})_r\text{C1-C4-alkyl}$, $-\text{OH}$, $-\text{NH}_2$, $-\text{NH}(\text{C1-C4-alkyl})$,
 $-\text{N}(\text{C1-C4-alkyl})_2$, $-\text{CO}_2\text{R}^4$, or
d) $-\text{C1-C4-alkylaryl}$, where aryl is defined above;

R^3 is H, alkyl, aryl, alkylaryl or an NH_2 -blocking group

15 comprised of 1-20 carbon atoms;

R^4 and R^5 are independently

- a) H,
b) C1-C4-alkyl, or
c) $-\text{CH}_2\text{-aryl}$, where aryl is defined above;

20 R^6 is

- a) H,
b) C1-C4-alkyl,
c) aryl, wherein aryl is phenyl or napthyl
optionally substituted with one or two substituents
selected from the group consisting of halo (F, Cl,
25 Br, I), C1-C4-alkyl, C1-C7-alkoxy, $-\text{NO}_2$, $-\text{CF}_3$,
 $-\text{S}(\text{O})_r\text{C1-C4-alkyl}$, $-\text{OH}$, $-\text{NH}_2$, $-\text{NH}(\text{C1-C4-alkyl})$,
 $-\text{N}(\text{C1-C4-alkyl})_2$, $-\text{CO}_2\text{R}^4$, or
d) $-\text{C1-C4-alkylaryl}$, where aryl is defined above;

30 A is an amino acid residue or a peptide comprised of 2-
20 amino acid residues;

Y^1 and Y^2 are

- a) $-\text{OH}$,
- b) $-\text{F}$,
- c) C1-C8-alkoxy, or

when taken together γ^1 and γ^2 form a
 d) cyclic boron ester where said chain or ring
 contains from 2 to 20 carbon atoms and, optionally,
 1-3 heteroatoms which can be N, S, or O,

5 n is 0 or 1;

p is 0 to 3;

q is 0 to 4;

r is 0 to 2;

and pharmaceutically acceptable salts thereof, with the
 10 proviso that when R^1 is aliphatic, an R^6 substituent on
 $-NHC(NH)NHR^6$ cannot be H.

2. A compound of claim 1 where

γ^1 and γ^2 are

a) -OH,

15 when taken together γ^1 and γ^2 form a

b) cyclic boron pinacol ester, or

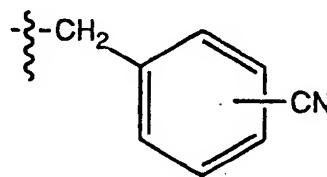
c) cyclic boron pinanediol ester;

R^1 is

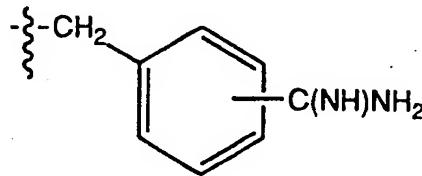
a) $-(CH_2)_3NHC(NH)H$,

20 b) $-(CH_2)_4C(NH)NH_2$,

c)



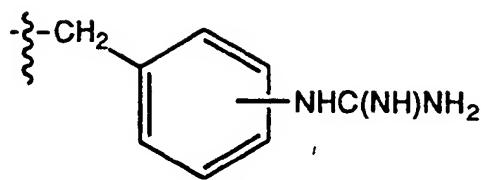
d)



25

, or

e)



R² is H;

A is Pro or (D)Phe-Pro;

R³ is

5 a) H,

b) Boc,

c) Z,

d) Ac,

e) hydrocinnamoyl,

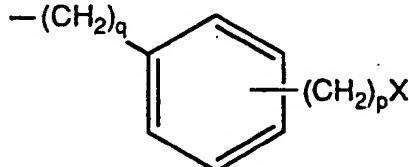
10 f) C1-C10 alkyl sulfonyl, or

g) C1-C15 alkylaryl sulfonyl.

3. A compound of claim 1 where

R¹ is

a)



15

4. A compound of claim 1 where

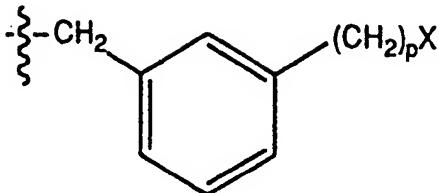
R¹ is

a) C3-C4-alkyl substituted with -CN, -C(NH)NH₂, -NH-

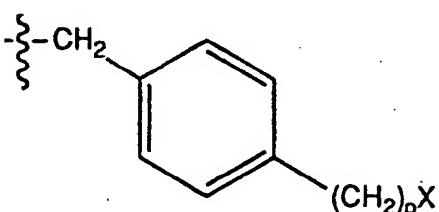
C(NH)H.

5. A compound of claim 1 where
R¹ is

a)



5 b)



X is

a) halogen (Cl, Br)

b) -CN,

10 c) -C(NH)NH₂,

d) -NH₂

e) -NHC(NH)NH₂;

p is 0 to 1.

6. A compound of claim 1 where R³ is H, alkyl, Ac, Boc,
15 Cl-C10 alkyl sulfonyl, Cl-C15 alkylaryl sulfonyl, Cl-C15
aryl sulfonyl.

7. A compound of claim 1 where n is 0.

8. A compound of claim 1 where [A] is comprised
independently of amino acid residues in the D or L
20 configuration selected from the group consisting of Ala,
Arg, Asn, Asp, Aze, Cys, Gln, Glu, Gly, His, Homolys,
Ile, Leu, Lys, Met, Orn, Phe, Phe(4-fluoro), Pro, Ser,
Thr, Trp, Tyr, and Val.

9. A compound of claim 1 where [A] is comprised of
25 either Pro or (D)Phe-Pro.

10. A compound of claim 1 selected from the group:

• Ac-(D)Phe-Pro-NH-CH[(CH₂)₄CN]BO₂-C₁₀H₁₆

- Ac- (D) Phe-Pro-NHCH[(CH₂)₄C(NH)NH₂]BO₂-C₁₀H₁₆
- Ac- (D) Phe-Pro-NHCH[(CH₂)₃-NHC(NH)H]B(OH)₂
- Boc- (D) Phe-Pro-NHCH[(CH₂)₃-NHC(NH)H]B(OH)₂
- Ac- (D) Phe-Pro-boroPhe[*m*-C(NH)NH₂]-C₁₀H₁₆
- 5 • Ac- (D) Phe-Pro-boroPhe(*m*-CH₂NH₂)-C₁₀H₁₆
- Ac- (D) Phe-Pro-boroPhe(*m*-Br)-C₁₀H₁₆
- Ac- (D) Phe-Pro-boroArg(CN)-C₁₀H₁₆
- Ac- (D) Phe-Pro-boroPhe(*p*-CN)-C₁₀H₁₆
- Boc- (D) Phe-Pro-boroPhe- (*m*-CN)-C₁₀H₁₆
- 10 • N,N-(CH₃)₂- (D) Phe-Pro-boroPhe- (*m*-CN)-OH•HCl (ISOMER I)
- Ac- (D) Phe-Pro-boroPhe- (*m*-CN)-OH•HCl
- Ms- (D) Phe-Pro-boroPhe- (*m*-CN)-OH•HCl
- Boc- (D) Thiazolylalanine-Pro-boroPhe- (*m*-CN)-C₁₀H₁₆
- 15 • Boc- (D) 3-Pyridylalanine-Pro-boroPhe- (*m*-CN)-C₁₀H₁₆
- Ms- (D) 3-Pyridylalanine-Pro-boroPhe- (*m*-CN)-C₁₀H₁₆
- Boc- (D) 2-Pyridylalanine-Pro-boroPhe- (*m*-CN)-C₁₀H₁₆
- Boc- (D) 2-Thienylalanine-Pro-boroPhe- (*m*-CN)-C₁₀H₁₆
- Ms- (D) 2-Thienylalanine-Pro-boroPhe- (*m*-CN)-C₁₀H₁₆
- 20 • Boc- (D) Phe-Aze-boroPhe- (*m*-CN)-C₁₀H₁₆
- Hydrocinnamoyl-Pro-boroIrg(CH₃)-OH•HBr
- Ac- (D) Phe-Pro-boroArg(CH₃)-OH•HCl
- PhCH₂SO₂- (D) Phe-Pro-boroOrn(CH=NH)-OH•HCl
- CH₃CH₂CH₂SO₂- (D) Phe-Pro-boroOrn(CH=NH)-OH•HCl
- 25 • CH₃CH₂CH₂SO₂- (D) Phe-Pro-boroArg(CH₃)-OH•HCl
- Ac- (D) Phe-Sar-boroOrn(CH=NH)-OH•HCl
- Boc- (D) Phe-Sar-boroPhe(*m*CN)-C₁₀H₁₆
- Boc- (D) Phe-Aze-boroOrn(CH=NH)-OH•HCl
- 4-(Phenyl)benzoyl-boroOrn(CH=NH)-C₁₀H₁₆•HCl

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/04058

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : A61K 37/02

US CL : 514/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/18

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS Online, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,499,082 (SHENVI ET AL.) 12 February 1985, see entire document.	1-10
A	US A, 4,537,773 (SHENVI) 27 August 1985, see entire document.	1-10

Further documents are listed in the continuation of Box C. See patent family annex.

• Special categories of cited documents:		
• "A" document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
• "E" earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
• "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
• "O" document referring to an oral disclosure, use, exhibition or other means	"A"	document member of the same patent family
• "P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

08 AUGUST 1994

Date of mailing of the international search report

AUG 18 1994

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Authorized officer

DAVID LUKTON

Faxsimile No. (703) 305-3230

Telephone No. (703) 308-0196